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Chemical Form of Cadmium (and Other Heavy Metals) in Rice and Wheat Plants

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Chemical forms of heavy metals such as Cd, Cu, Ni, and Pb in rice and wheat plants grown in nutrient solution containing a heavy metal were investigated. Fractionation of an extract of Cd-treated rice plants on Sephadex G-75 showed cadmium to be associated with organic compounds of high (fraction A), intermediary (fraction B), and low molecular weight (fraction C). Material A, whose molecular weight was greater than 440,000, is probably nonspecific binding of Cd to normal cell components. Materials B and C can be classified as types of metallothionein. The molecular weight of B was 33,100. This material contains 12 mg Cd/g protein. The UV-absorption spectrum of B showed absorptions at 280 and 250 nm. Material B was not eluted even at a very high ionic strength from the DEAE-cellulose column, but it was eluted at a very low ionic strength from a CM-cellulose column, indicating a highly anionic molecule which differs from metallothionein in animals. Fraction C contains two materials: one a Cd-containing material whose molecular weight was estimated to be approximately 7000 and the other an inorganic Cd salt. In addition to cadmium, copper, lead, and nickel in rice and wheat have been studied. As a result, heavy metal-containing materials whose molecular weights were estimated to be approximately 16,000 and 8900 (Ni-treated rice plants), 7000 (Pb-treated rice plants), 5000 (Cd-treated wheat plants), and 21,000 (Cu-treated wheat plants) were isolated.

Introduction

Human cadmium toxicity caused by Cd-contaminated rice plants was first reported in Japan in the 1950s (*1*). In this case, the source of excess cadmium came from rice plants grown on paddy fields contaminated with cadmium by a zinc-mining operation. Since rice and wheat are staple foods of the Japanese people, Cd contamination of rice and wheat (as well as environmental Cd contamination) is a serious problem for human health in Japan. Cadmium poisoning of humans in our country has stimulated research on cadmium and other heavy metals in rice and wheat plants. Because the constitution of heavy metal compounds formed in plants for food may be an important factor in human heavy metal toxicity, we have been investigating the distribution and chemical form of cadmium (and other heavy metals such as copper, lead, and nickel) in rice and wheat plants (*2*).

Chemical Form of Cadmium in Rice Plants

Seeds of "nihonbare" rice plant (*Oryza sativa* L) were germinated in soil and transplanted as 4-week-old seedlings into an aerated nutrient solution containing 88.3 mg/L KH_2PO_4; 35.7 mg/L NH_4NO_3; 203.0 mg/L $MgSO_4$ $7H_2O$; 73.5 mg/L $CaCl_2$; 22.5 mg/L EDTA·FeNa·$2H_2O$: and the trace element B (0.5 ppm), Mn (0.05 ppm); Cu (0.03 ppm) ; Zn (10.05 ppm), Mo (0.02 ppm) and Co (0.02 ppm). The initial pH of the nutrient solution was 5.5. After 2 weeks, the plants received an application of $CdCl_2$ at a concentration of 0.3 mg Cd^{2+}/L for 1 month. The plants were harvested, divided into roots and stems, rinsed with deionized water, cut into small pieces, and homogenized in 0.05 M sodium phosphate buffer, pH 7.8, in a chilled electric blender. The homogenate was extracted with the same buffer for 1 hr with continuous stirring and stored at 4°C for 2 weeks. The homogenate was squeezed through cloth. The filtrate was lyophilized and centrifuged at 5000 rpm for 30 min. The supernatant fluid was applied to a Sephadex G-75 column (5.4 × 120 cm). Elution was carried out with 0.05 M sodium phosphate buffer, pH 7.8, at a flow rate of 30 mL/hr. The gel filtration of the supernatant on Sephadex G-75 resulted in three main cadmium peaks (Fig. 1.). Cadmium in the rice plant was found to be associated with organic compounds of high, intermediate, and low molecular weight, shown as fractions A, B, and C in Figure 1.

Fraction B of Figure 1 was concentrated by ultrafiltration on a UP-20 (TOYO) and was rechromatographed on a Sephadex G-50 column as shown in Figure 2. The cadmium-rich fractions after concentration and lyophili-

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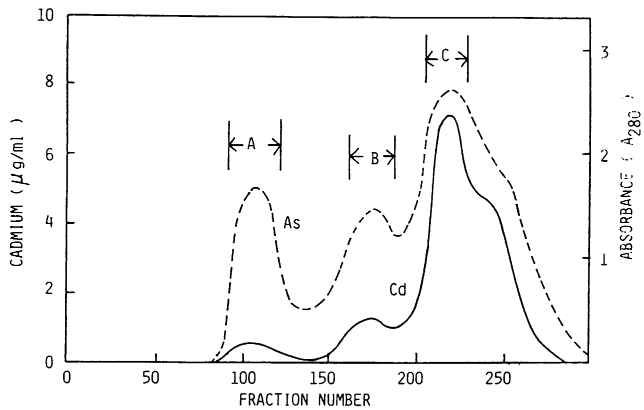

FIGURE 1. Sephadex G-75 elution profile of extract from cadmium-treated rice plants. The extract was chromatographed on a column (5.4 × 116 cm) equilibrated with 0.05 M sodium phosphate buffer, pH 7.8. Fractions (10 mL) were collected at a flow rate of 30 mL/hr.

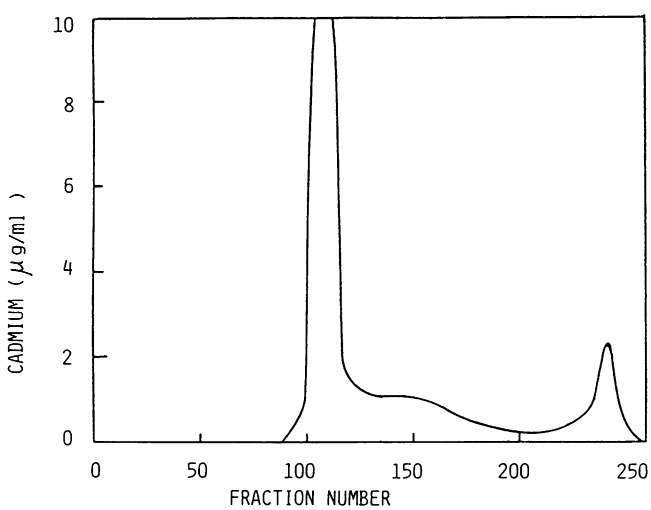

FIGURE 2. Rechromatography of fraction B (Fig. 1) on a Sephadex G-50 column. Fraction B from the Sephadex G-75 column (Fig. 1) was rechromatographed on a Sephadex G-50 column (5.4 × 118 cm) equilibrated with 0.05 M sodium phosphate buffer, pH 7.8. Fractions (10 mL) were collected at a flow rate of 30 mL/hr.

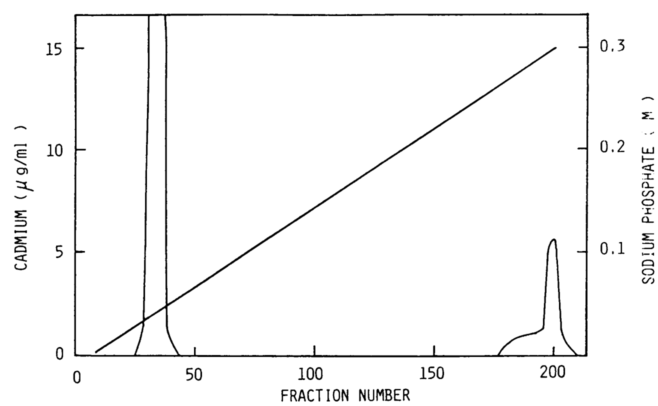

FIGURE 3. Chromatography of the cadmium-rich fraction from Fig. 2 on a CM-cellulose column. The cadmium-rich fraction from Fig. 2 from the Sephadex G-50 column was applied to a CM C-50 cellulose column (2.9 × 92 cm) equilibrated with 0.01 M sodium phosphate buffer, pH 7.0. Elution was carried out with a linear gradient of 0.01 to 0.3 M sodium phosphate buffer, pH 7.0. Fractons (10 mL) were collected at a flow rate of 20 mL/hr.

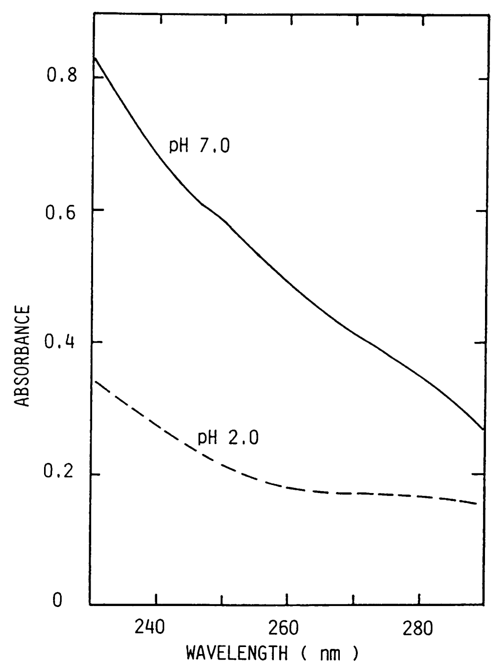

FIGURE 4. Ultraviolet absorption spectrum of a cadmium-binding protein from fraction B of Fig. 1: absorption spectra at (——) pH 7.0 and (--) pH 2.0 (adjusted with HCl).

zation were purified further by passage through a CM–cellulose column as shown in Figure 3. Further purification was performed by column electrophoresis. The cadmium-containing material from the CM–cellulose column was mixed with 3% saccharose and separated on a 6 × 10 cm polyacrylamide gel column, equilibrated with 0.02 M sodium phosphate buffer, pH 7.8, at 10°C and 35 mA. After 40 hr of electrophoresis, 5 mL fractions were collected at a flow rate of 10 mL/hr. The cadmium-containing material moving toward the anode was recovered in a single peak. Homogeneity of the purified cadmium-containing material was evident on analytical polyacrylamide-disc gel electrophoresis. This material contains 12 mg·Cd/g protein. It reacts positively to the biuret and ninhydrin tests. The ultraviolet absorption spectrum of this material showed absorptions at 280 and 250 nm (Fig. 4). The absorption at 250 nm was significantly reduced upon acidification to pH 2. This observation suggests that this material is a cadmium-binding protein like metallothionein. By means of gel-permeation chromatography, the molecular weight of the material was estimated to be 33100 (Fig. 5), which differs from that of metallothionein in animals. This material was not eluted even at a very high ionic strength from the DEAE–cellulose column. But it was eluted at a very low ionic strength from a CM–cellulose column, indicating a highly anionic molecule. On the basis of these results, this cadmium-containing material isolated from cadmium-treated rice

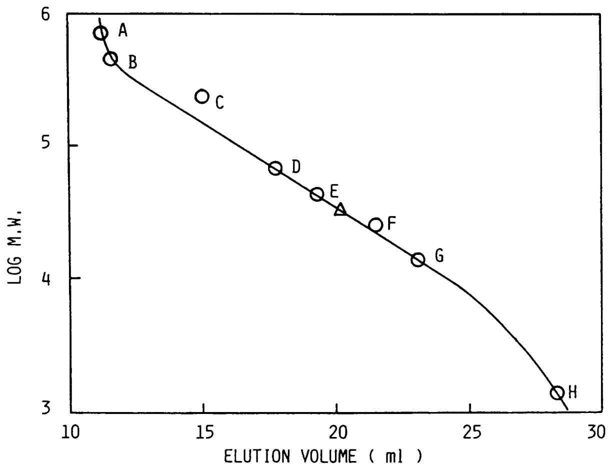

FIGURE 5. Determination of molecular weight of cadmium-binding protein in fraction B of Fig. 1. The molecular weight of a cadmium-binding protein (Δ) was determined by gel-permeation chromatography on a TSKG 3000 SW column (0.75 × 60 cm). The column was equilibrated with 0.2 M sodium chloride, 1/30 M sodium phosphate buffer, pH 7.0. The flow rate was maintained at 1 mL/min. The effluent fractions were monitored at 280 nm to determine the elution volume of proteins. The proteins used as standards were: (A) thyroglobulin (MW 660,000); (B) ferritin (MW 440,000); (C) catalase (MW 210,000); (D) albumin (MW 67,000); (E) ovalbumin (MW 43,000); (F) chymotrypsinogen A (MW 25,000); (G) ribonuclease A (MW 13,700); (H) bacitracin (MW 1400).

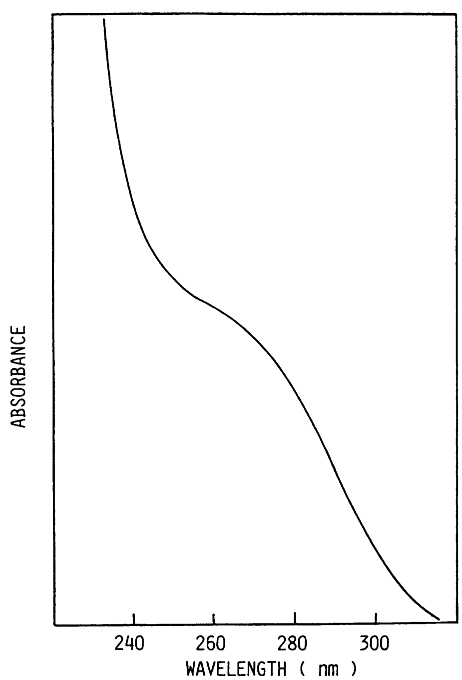

FIGURE 6. Ultraviolet absorption spectrum of cadmium-binding material in Fraction A of Fig. 1.

plants can be classified as a type of metallothionein which differs from that in animals (*3–9*). Characterization of this material in detail is in progress.

Fraction A of Figure 1 was purified further by rechromatography and column electrophoresis and was

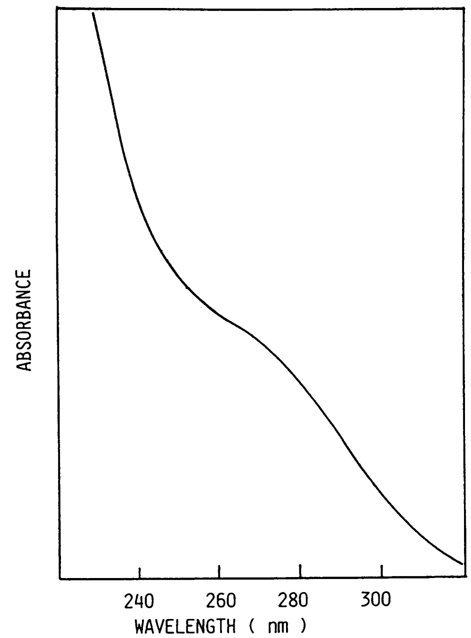

FIGURE 7. Ultraviolet absorption spectrum of cadmium-binding material in Fraction C of Fig. 1.

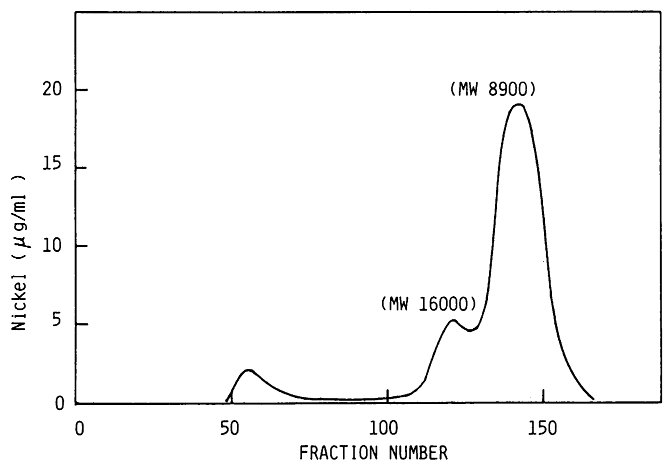

FIGURE 8. Sephadex G-75 elution profile of extract from nickel-treated rice plants.

analyzed for molecular weight. The molecular weight was estimated to be greater than 440,000. The material is probably nonspecific binding of cadmium to normal cell components (Fig. 6).

Fraction C of Figure 1 seemed to be a major cadmium-containing component in the rice plant. It was applied to a CM–cellulose column equilibrated with 0.01 M sodium phosphate buffer, pH 7.4. Elution was carried out with a linear gradient of 0.01 to 0.3 M sodium phosphate buffer. As a result, fraction C was separated into two Cd-containing fractions. One Cd-containing material fraction was eluted at an ionic strength of 0.01 M sodium phosphate buffer. The molecular weight of this material was estimated to be approximately 7000. Characterization of this material is in progress. The other Cd-

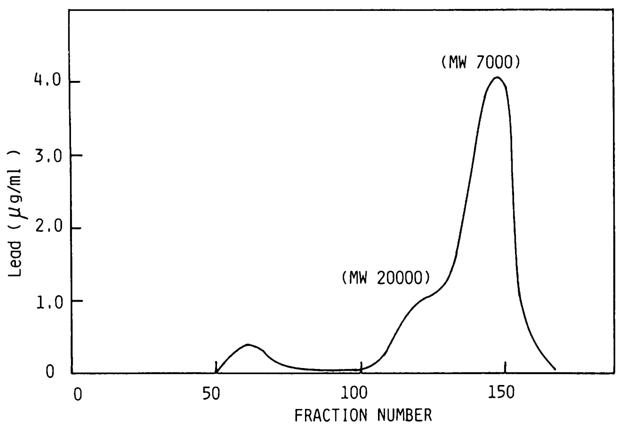

FIGURE 9. Sephadex G-75 elution profile of extract from lead-treated rice plants.

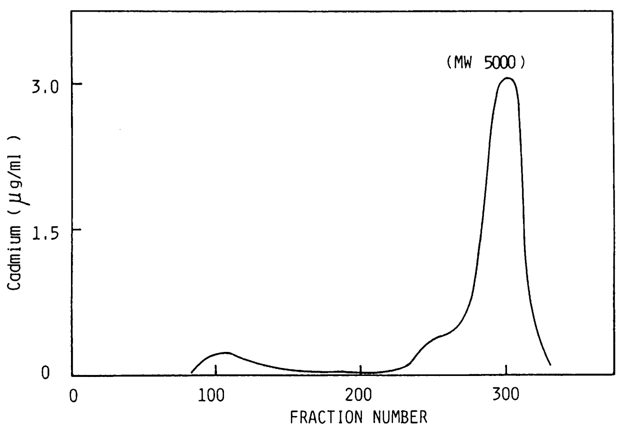

FIGURE 10. Sephadex G-75 elution profile of extract from cadmium-treated wheat plants.

containing material in fraction C which was eluted at an ionic strength of 0.3 M sodium phosphate buffer was subjected to Sephadex G-25 column chromatography (Fig. 7). This material is associated with very low molecular weight material with the ratio of elution volume to column void volume (V/V_o) of 2.2. This material probably is an inorganic cadmium salt.

Heavy Metals in Rice and Wheat Plants

In addition to cadmium, we studied copper, lead, and nickel in rice and wheat plants. The chemical form of copper and nickel in plants has recently been reported by several investigators (*10–13*).

All experimental procedures for growing plants, extracting, and isolating metal-containing components were similar to those used in the experiments on cadmium in rice plants. Sephadex G-75 elution profiles of the extracts from each plant are shown in Figures 8–12. The molecular weights of metal-binding materials were estimated to be approximately 16,000 and 8900 (Ni-treated rice plants), 7000 (Pb-treated rice plants), 5000 (Cd-treated wheat plants), and 21,000 (Cu-treated wheat plants) respectively. Characterization of these materials is in progress.

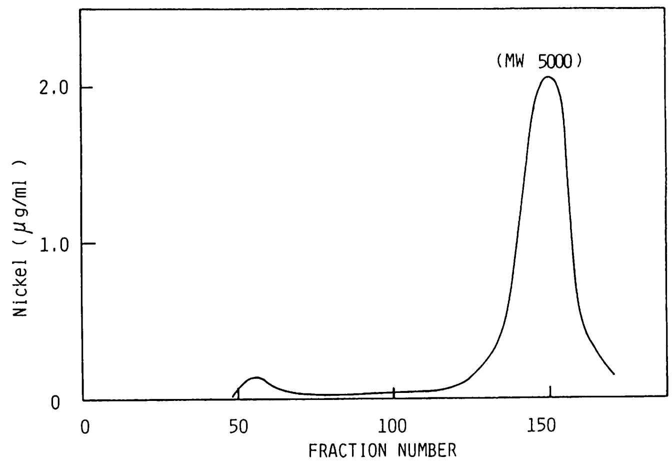

FIGURE 11. Sephadex G-75 elution profile of extract from nickel-treated wheat plants.

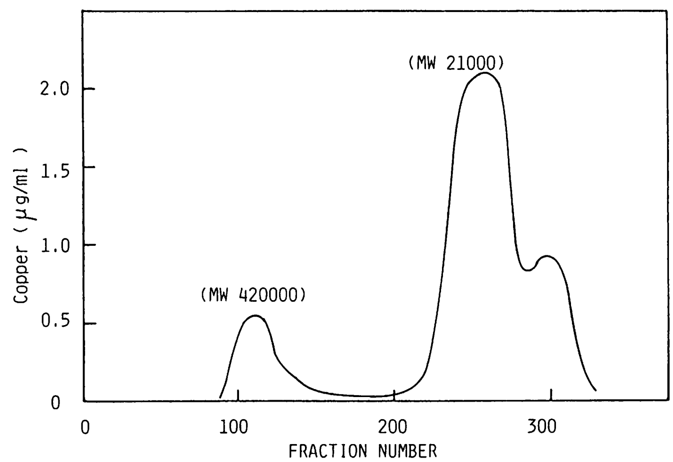

FIGURE 12. Sephadex G-75 elution profile of extract from copper-treated wheat plants.

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